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(54) Method, test strain and test kit for the laboratory diagnosis of neisseria gonorrhoeae.

(57) A strain of *Neisseria gonorrhoeae* ATCC 31953 is described which has characteristically poor growth on chocolate agar at a temperature range of about 30°C to about 37°C in a CO_2 atmosphere suitable for growth of *N. gonorrhoeae*. This strain is resistant to nalidixic acid at the 5-10 mcg/ml level and resistant to streptomycin at the 1000 mcg/ml level or greater. The strain is suitable for a method for the laboratory diagnosis of gonorrhea, comprising the steps of (1) preparing a test strain of the microorganism *Neisseria gonorrhoeae* which is competent for transformation, (2) extracting *N. gonorrhoeae* DNA from a patient's specimen material or colonies suspected of being *N. gonorrhoeae* whether in pure or mixed culture by treatment with a base which lyses *N. gonorrhoeae*, (3) adusting the pH of the extract of step (2) to a pH that is not toxic to the test strain, (4) applying the pH adjusted extract to the test strain before or after putting it on or into a biological medium suitable for growth of *N. gonorrhoeae*, (5) maintaining the treated test strain at optimum *N. gonorrhoeae* growth conditions which are inhibitory for the test strain and (6) observing for detectable growth of the treated test strain. The observance of growth indicates detection of *N. gonorrhoeae* DNA. Also described is a test kit for performing the method.

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BRIEF SUMMARY OF THE INVENTION

The standard laboratory diagnosis of gonorrhea depends on isolation and subsequent identification of Neisseria gonorrhoeae by colony morphology, microscopic examination, biochemical or serologic tests (Kellog, Jr., et al., Laboratory Diagnosis of Gonorrhea, Cumitech, Amer. Soc. Microbiol., 1976). Although gram stain examination can be used to diagnose gonococcal urethritis, this technique lacks sensitivity in detecting infections of the cervix, rectum or oropharynx. Other diagnostic methods, such as serologic testing or direct fluorescent antibody staining, have not proven useful.

Because N. gonorrhoeae loses viability rather quickly, the best procedure for isolating gonococci is to culture a specimen as soon as possible. This requires special facilities since it is necessary to incubate the inoculated culture media at $35^{o}C$-$37^{o}C$ in a CO_2 atmosphere. When proper facilities are not available, specimens can be sent to a laboratory in Amies' or Stuart's transport medium or in a transport and growth medium such as Transgrow (Martin, Lester, HSMHA Health

rep. 86:30, 1971). The latter procedures are not nearly as good as immediate culturing which itself is about 90-95% sensitive in detecting gonococci (Schmaly, Martin, Domescik, J.Am.Med. Assoc. 210:312, 1969; Caldwell, Price, Pazin, Cornelius, Am. J. Obstr. Gynecol. 109:463, 1971).

In 1976, a transformation test was reported which could be used to identify a clinical isolate as N. gonorrhoeae and thereby to help diagnose gonorrhea (Janik, Juni, Heym, J. Clin. Microbiol. 4:71; Juni U.S. Patent No. 3,930,956). A transformation test depends on detecting gonococcal DNA as compared to a culture technique which depends on isolating colonies. In preliminary laboratory studies, the test appeared to be a useable alternative to standard procedures for identifying colonies of N. gonorrhoeae (Bawdon, Juni, Britt, J. Clin. Microbiol. 5:108, 1977; Sarafian, Young, J. Med. Microbiol. 13:291, 1980). However, in a field trial, done in collaboration with the Centers for Disease Control, using specimens obtained from clinic patients, the test described was found to be insensitive and nonspecific for the laboratory diagnosis of gonorrhea infections.

The Juni transformation test depends on DNA from gonococci in a colony or a specimen, to contain genes which can correct a nutritional deficiency of a test strain of N. gonorrhoeae, i.e. the test strain cannot grow on a special test medium unless it is given a particular nutrient. However, this strain can take up and incorporate gonococcal DNA (genes) into its genome, so that it is transformed with such genes and can now grow on the special test medium. If the gonococci of a colony, or in a specimen, and the test strain happened to have the same nutritional deficiency, then the test strain cannot be transformed to grow on the special test medium, thereby preventing detection of N. gonorrhoeae DNA from a colony or specimen. Since it is known that there is a variety of

nutritional variants of N. gonorrhoeae which infect humans (Crawford, Sex. Trans. Dis. 5:165, 1978), the Juni test obviously requires a battery of test strains with different nutritional deficiencies in order to cover the spectrum of possibilities in any one test situation.

The Juni test requires the use of two culture media, one of which is so specialized that it is not available in a routine diagnostic laboratory. The technique for extracting DNA calls for using surfactants such as sodium dodecyl sulfate at a concentration which is at the borderline level of toxicity for the test strains. This could negate potentially positive results. The transformation technique involves a preliminary incubation period of the DNA and the test strain, followed by a second step which involves subculturing the DNA-test strain mixture onto the specialized medium. After 24 hours of incubation the results of their test are barely visible to the naked eye. One needs a microscope to detect the growth of colonies which indicates a positive result (Bawdon et al, J. Clin. Microbiol. 5:108, 1977).

The present invention relates to a novel test strain N. gonorrhoeae ATCC 31953, which when utilized in accordance with the novel method of this invention, enables an accurate laboratory diagnosis of gonorrhea to be made by detecting N. gonorrhoeae DNA. The test strain, N. gonorrhoeae ATCC 31953, has been deposited with the American Type Culture Collection, Rockville, MD, in accordance with Rule 28 EPC. This novel test strain barely grows in conditions considered to be optimmum for N. gonorrhoeae and has certain antibiotic resistance genes introduced by genetic recombination.

The method of this invention comprises the steps of (1) preparing a test strain of the microorganism Neisseria gonorrhoeae which is competent for

transformation, (2) extracting N. gonorrhoeae DNA from a patient's specimen material or colonies suspected of being N. gonorrhoeae whether in pure or mixed culture by treatment with a base which lyses N. gonorrhoeae, (3) adjusting the pH of the extract of step (2) to a pH that is not toxic to the test strain, (4) applying the pH adjusted extract to the test strain before or after putting it on or into a biological medium suitable for growth of N. gonorrhoeae, (5) maintaining the treated test strain on or in a medium suitable for growth of N. gonorrhoeae at optimum N. gonorrhoeae growth conditions which are inhibitory for the test strain and (6) observing for detectable growth of the treated test strain. The observance of growth indicates positive detection of N. gonorrhoeae DNA.

In accordance with this invention a test kit is provided which enables the method of this invention to be performed, which kit includes a test strain of N. gonorrhoeae which grows poorly at about 36°C on or in a biological medium, and may optionally contain other components required to perform the test such as a biological medium capable of supporting growth of a test strain, a base, an acid, a pH indicator, and necessary laboratory equipment and supplies.

The novel method of this invention requires only one test strain. It depends on a genetic characteristic common to all N. gonorrhoeae causing human infections, namely, the ability to grow at about 36°C. The invention permits the use of such biological media as common chocolate agar. Extracting gonococcal DNA by the use of base and adjusting the pH of the extract to a pH tolerated by the test strain with an acid results in a salt which is not toxic to the test strain. The procedures are very simple. Positive results are seen in 27-40 hours without the use of a microscope. In a field

trial conducted in collaboration with the Centers for Disease Control, the method of this invention was found to be as sensitive and specific as the culture technique for the diagnosis of gonorrhea.

DETAILED DESCRIPTION OF THE INVENTION

I. Test Strain, N. gonorrhoeae ATCC 31953

The preferred test strain of N. gonorrhoeae for use in the method of this invention, N. gonorrhoeae ATCC 31953 grows poorly on chocolate agar within a temperature range of from about 30°C to about 37°C, in a CO_2 atmosphere. Specifically, this strain barely grows in conditions considered to be optimum for N. gonorrhoeae i.e., on common chocolate agar, at a temperature around 36°C in a CO_2 atmosphere.

This strain is also resistant to nalidixic acid at the 5-10 mcg/ml level and resistant to streptomycin at the 1000 mcg/ml level or greater. Details of the mutation and the genetic recombination procedures which resulted in the isolation of N. gonorrhoeae ATCC 31953 follow.

The chemical mutagen, N-methyl-N'-nitro-N-nitrosoguanidine, was added at a final concentration of 25 mcg/ml to GC buffer (an aqueous solution of 1.5% peptone, such as Proteose Peptone, Difco, Detroit, Michigan, 0.028 M potassium phosphate, dibasic and 0.007 M potassium phosphate, monobasic) which contained about 10^9 cells of a common laboratory strain of N. gonorrhoeae prepared from colony types 1 or 2 (Kellogg, Peacock, Deacon, Brown, Pirkle, J. Bacteriol. 85:1274, 1963). After a 30 minute incubation of the mutagen - cell suspension in a water bath set for 37°C, the suspension was centrifuged to pellet the cells. The supernate was removed to discard the mutagen. The pelleted cells were resuspended in GC buffer. After centrifugation a second time, the pellet

was again resuspended in GC buffer. The suspension was then plated on GC agar (in petri plates), which is GC medium base (GC buffer, 0.5% NaCl, 0.1% cornstarch, 1.0% agar) supplemented at a final concentration in gms/100ml, with glucose 0.4g, glutamine 0.01g, cocarboxylase 0.00002g, $Fe(NO_3)_3 \cdot 9H_2O$ 0.0005g. The petri plates were incubated overnight at 30°C and then switched to 37°C for the second night of incubation. Small colonies were picked and tested for the inability to grow luxuriously at 37°C on GC agar. A poor growing variant of N. gonorrhoeae judged to be potentially useful in the method of the invention was isolated in this manner and used to obtain the test strain having specific antibiotic resistance. The antibiotic resistance genes were introduced into this variant in the following manner.

DNA was extracted from the RW-2 strain of N. gonorrhoeae which contains the Nal gene conferring resistance to nalidixic acid at the 5-10 mcg/ml level (Wharton, Zubrzycki, J. Bacteriol. 127:1579, 1976). The DNA extraction procedure was accomplished by adding NaOH at a final concentration of 0.1N to a suspension of RW-2 (containing approximmately 1×10^8 cells/ml) in GC buffer. The extract was then neutralized with HCl.

Two-tenths ml of this neutralized extract containing RW-2 DNA was added to a 1.8 ml suspension of the variant (about 1×10^8 cells/ml) in GC buffer which contained approximately 10^{-3}M $CaCl_2$. After incubating the DNA-cell mixture for 30 min. in a water bath set for 30°C, 0.1ml aliquots of the mixture were put onto, and then evenly spread on the surface of six petri dishes containing GC agar. After incubating the plates in a candle extinction jar for 4 hours, the GC agar was lifted from the petri dish and placed on top of another layer of GC agar which contained 10mcg/ml Nal. This double-layered agar, with the DNA-cell mixture on the top, was incubated

for about 40 hours at 30°C in a CO_2 atmosphere. Of the many colonies which appeared, presumably resistant to Nal, twenty were selected at random and subcultured. Of these, one was selected as being best suited, at this time, for use in the method of this invention.

Using essentially the same procedures as just described, DNA from N. gonorrhoeae strain 24392 (Maier, Zubrzycki, Coyle, Antimocrob. Ag. Chemo. 7:676, 1975) was used to introduce the Str gene into the Nal variant. Of 14 isolates which were resistant to both antibiotics, Nal at the 5-10 mcg/ml level and Str at the 1000mcg/ml level or greater, one was selected as being best suited as the test strain for use in the method of this invention, N. gonorrhoeae ATCC 31953. The test strain N. gonorrhoeae ATCC 31953 has morphological and biochemical characteristics generally like those of Neisseria gonorrhoeae.

II. Growth and Preparation of a Test Strain

A test strain is prepared for use in accordance with the method of this invention by growing it on the surface of GC agar at 30°C in a CO_2 atmosphere suitable for N. gonorrhoeae, for example: a candle extinction jar; a CO_2 incubator; a CO_2 generating system such as a Gas-Pak system (Baltimore, Biological Laboratories, Cockeysville, Maryland). After overnight incubation, areas of colony growth on the agar plates having mostly colony types 1 and/or 2 (Kellogg, Peacock, Deacon, Brown, Pirkle, J. Bacteriol. 85:1274, 1963) are harvested with sterile cotton swabs which are inserted into 10-20 ml volumes of GC buffer containing 10-20% glycerol, in order to make a suspension equivalent to a number 4 or 5 McFarland standard (Lennette, Spaulding, Truant, Manual of Clinical Microbiol., Amer. Soc. Microbiol., p. 933, 1974). Selection of colony types 1 or

2 is required to ensure that a test strain in a preparation is at the optimum state of competence, i.e. the state of optimum uptake of DNA which is necessary for transformation. Five ml volumes of this suspension are put into tubes which in turn are put into a -70°C freezer and stored until needed. When needed for the method of the invention, the suspension is thawed by placing the tube into a 37°C H_2O bath.

A freshly prepared suspension of a test strain can also be used. In this case, the turbidity of the suspension can be anywhere between a quarter of a number 1 McFarland up to a number 4. The suspending medium need not contain the glycerol. The frozen-thawed and the fresh are the preferred preparations of a test strain for use in accordance with the method of this invention.

A third alternative is to use a suspension made from a lyophilized preparation of a test strain. A suspension at a turbidity of a McFarland 5 or 6 is made in a medium which contains approximately: 1.5% peptone; 0.04% L-glutamic acid; 5% albumin; 5% serum; 5% glycerol; 0.2% starch; 0.4% glucose; 10% sucrose. This medium is adjusted with KOH to a pH of about 7.2. The suspension is then lyophilized.

The lyophilized preparation is reconstituted in GC buffer and used immediately or reconstituted in GC buffer containing the following growth supplements in gms/100 ml: glucose 0.4g, glutamine 0.01g, cocarboxylase 0.00002g, $Fe(NO_3)_3 \cdot 9H_2O$ 0.0005g. In the latter case, the reconstituted suspension is incubated for 4-6 hours at 30°C in a CO_2 atmosphere before use. The use of the lyophilized test strain after incubation gives better results, i.e., more colonies in a positive test than the direct use of the lyophilized preparation.

While reference is made to growing a test strain on the surface of a medium containing agar, specifically

GC agar, this does not exclude growing a test strain on or in any medium. For example, a liquid culture in a flask or fermenter may be used. Although reference is made to using GC buffer with or without glycerol, this does not exclude using any other medium suitable for maintaining the viability of a test strain, fresh or frozen. Also while reference is made to the special medium in which a test strain is lyophilized, this does not exclude using any other medium suitable for lyophilizing a test strain. When a test strain is resistant to an antibiotic, this antibiotic can be added to any of the liquid or solid media used in preparing the test strain for use. For example, Nal at 5 mcg/ml and Str at 1000 mcg/ml were added to a frozen lot of the test strain, N. gonorrhoeae ATCC 31953, in order to rid it of a contaminant when a lot was subsequently used. Now, as a precaution, these antibiotics are routinely added to the GC buffer with or without glycerol used in preparing a suspension of the test strain, N. gonorrhoeae ATCC 31953, and are added to the medium in which the test strain is lyophilized.

III. The Extraction and Neutralization

The preparation of a DNA extract involves placing a patient's specimen material or suspect colonies into a base which lyses the gonococci and then adjusting the pH of the extract with acid to a pH which is not toxic to a test strain. A variation is to make a suspension of the specimen material or colonies in GC buffer and then adding the base and acid to that suspension. In either case, the prepared extract is generally heated. The preferred base and acid are NaOH and HCl.

NaOH and HCl solutions are prepared by diluting commmercially available 10N or 1N NaOH solutions and 37%-38% or 1N HCl solution in GC buffer. Diluting the NaOH and HCl in this buffer is an important procedure.

The buffering effect allows one to use a little more or less of the recommended volumes of the NaOH and HCl without changing the pH below 6.0 or above 8.0. This range of pH is tolerated by the test strain in the transformation test to be performed. KOH or even NH_4OH can be used instead of NaOH. It is expected that any base can substitute for NaOH, even a weak organic base. It is expected that any acid can be used to offset the base, even weak organic acids. In each case, the principle is the same, namely, a base is used to lyse gonococci to release DNA, and an acid is used to adjust pH of the extract to a pH which is not toxic to a test strain.

The volumes of GC buffer, NaOH and HCl can vary. Some of the combinations which were found to be useful are as follows: 0.2-0.3 ml 0.1N NaOH and 0.3-0.4 ml 0.05N HCl; 0.5 ml 0.1N NaOH and 0.6-0.8 ml 0.05N HCl; 0.5 ml GC buffer, 0.1-0.2 ml 0.5N NaOH and 0.2 ml 0.2-0.4N HCl; 0.5 ml GC buffer, 0.1 ml 0.4N NaOH and 0.1-0.2 ml 0.2N HCl. The principle is to keep the volumes of GC buffer, NaOH and HCl small enough to minimize dilution of the DNA and yet large enough for convenient use in a routine laboratory situation. Again, it should be noted that GC buffer is not required as the suspension medium although it is used in the sample extraction procedure for the cervical swab specimen hereinafter described.

As an aide to seeing that the prepared DNA extract is near a neutral pH, phenol red at a final concentration of 0.005% is used in the base, acid, GC buffer or even all three. This does not exclude using it at any other useable concentrations. Nor does this exclude using other acid-base pH indicators for the purpose just stated.

After the base-acid extraction procedure, the extract is heated to 60°C-70°C for 10 min - 15 min. Temperatures as high as 75°C and time periods as long as

30 min. have been used without any apparent effect on the results. With pure cultures of N. gonorrhoeae, the heating step is not necessary because gonococci are very suceptible to treatment with a base. This is not the case with some other organisms found in mixed cultures and clinical specimens. Heating inactivates those organisms which escape treatment with a base.

As a further precaution against contamination during or after extracting a specimen or colonies, an antibiotic tolerated by a test strain is added to the GC buffer. An antibiotic can be added to the base and acid as well. An antibiotic can similarly be added to any of the media useful in accordance with the method of this invention. When using the test strain, N. gonorrhoeae ATCC 31953, Nal and Str are the antibiotics used at concentrations of 5 mcg/ml and 1000 mcg/ml, respectively. The concentrations of antibiotics stated does not exclude using these antibiotics at higher concentrations tolerated by the test strain.

Ethyl alcohol at a final concentration of about 70% can be added to the pH adjusted DNA extract whether it has been heated or not. The use of alcohol may be preceded by an addition of about 0.01% albumin to an extract. The albumin acts as an inert carrier for the precipitate which occurs. The suspension is centrifuged at low speed to pellet the precipitate which contains DNA or the precipitate can be collected on filters. The precipitate is dissolved in a small amount of GC buffer, which may contain antibiotics tolerated by a test strain. The amount of GC buffer added is one-tenth to two-tenths the original volume, resulting in a concentrated extract. After about 10 minutes at room temperature, the concentrated extract is useable in the transformation step. The use of a concentrated extract usually yields more colonies in a positive test than the use of an extract which is not concentrated.

Ethyl alcohol can also be used to concentrate the DNA while it is being extracted by the base. In this case, an alcohol-base solution extracts and precipitates the DNA simultaneously. Again the addition of albumin is optional. The precipitate can be harvested after centrifugation or after collection on a filter. In both cases the suspending medium, GC buffer, should be at a pH of around 6.0 to offset any residual base in order that the final concentrated DNA solution would not be above pH 8.0.

A virtue of using an alcohol procedure is that the alcohol acts as a germicide to inactivate viable organisms. In most situations, this would eliminate the need for the heating step in the extraction procedure described above.

IV. A Sample Extraction

A sample extraction procedure is now being presented. A cervical swab is put into a test tube which contains 0.5ml GC buffer. The cervical swab is mixed in the buffer and left in the tube for the remainder of the extraction procedure or, squeezed against the side of the tube and discarded. To the resultant suspension of mucus and cells is added 0.2 ml of 0.3N NaOH (containing 0.005% phenol red). After a brief time (approximately 30 seconds) 0.2ml to 0.3ml of 0.2N HCl is added to the extract. The test tube is then heated for 10 minutes in a water bath set at 68°C. After cooling to room temperature, the extract is ready for the transformation step of this invention. This example does not exclude using rectal, urethral or other specimens. The same extraction procedure can be used on colonies suspected of being N. gonorrhoeae from pure or mixed cultures, picked by an applicator stick, inoculating loop, needle or any implement made of wood, plastic, metal or paper as long as

it can be used to deliver gonococci into the GC buffer or base solution.

V. The Transformation

The transformation step of the test method of this invention is carried out by applying the prepared DNA extract to a test strain which grows poorly at about 36°C on or in a biological medium which can contain antibiotics at concentrations tolerated by the test strain. The preferred method of applying the extract to a test strain is by spotting it onto a lawn of the test strain which is prepared on a common chocolate agar plate. To prepare this lawn preferably two sterile cotton swabs held side by side are dipped into a suspension of a test strain. These swabs are then evenly smeared on the surface of a chocolate agar plate in a manner similar to that done for an antibiotic disk susceptibility test (Lennette, Spaulding, Truant, Manual of Clinical Microbiology, Amer. Soc. Microbiol., p. 423, 1974). One can use a single swab or any number of swabs, or any other implement or method for seeding a chocolate agar plate with a lawn of a test strain. Although a commercial chocolate agar plate (Baltimore Biological Laboratories, Cockeysville, Maryland) has been used in many of my studies, this does not exclude any other useable nutrient agar or chocolate agar whether in a petri dish, any type of container or on any surface. This does not exclude a nutrient agar or chocolate agar or any useable growth medium which contains antibiotics at concentrations which are tolerated by a test strain and on which the test strain grows poorly at a temperature around 36°C in a CO_2 atmosphere. This does not exclude a medium containing an ingredient which can substitute for CO_2 such as $NaHCO_3$.

After the lawn of a test strain is spotted with the extract, the chocolate agar plate is incubated at a temperature around 36°C in a CO_2 atmosphere suitable for growth of N. gonorrhoeae. After 27-40 hours incubation, a positive test is seen as an area of colony growth where the extract was spotted. A negative test shows no area of colony growth at the spotted area.

Although is is preferred to use a test strain in the form of a lawn, it is within the scope of this invention to use a test strain in any manner in which gonococcal DNA can be used to stimulate detectable growth of a test strain under conditions which are inhibitory for example, prohibitive temperatures. This means that the test could be run in a liquid environment, with or without antibiotics tolerated by a test strain, containing the test strain and gonococcal DNA as long as the presence of that DNA accounts for the growth of the test strain which would not take place without the gonococcal DNA.

VI. Supporting Data

The efficacy of the test method in accordance with the method of this invention was shown in a blind study done in collaboration with the Centers for Disease Control in which the results of the method of this invention were compared to clinical culture analyses. Cervical and rectal specimens from women and urethral specimens from men were collected at a DeKalb County, Georgia, clinic. Two swabs were taken from each anatomic site: one was immediately plated on selective medium and processed at the clinic; the other was mailed to Philadelphia, Pennsylvania where the test in accordance with the method of this invention was carried out. The following positive (+) and negative (-) results were obtained:

METHOD OF INVENTION	CULTURE METHOD					
	CERVICAL		RECTAL		URETHRAL	
	+	-	+	-	+	-
+	69	2	26	4	74	2
-	2	132	1	128	2	66

With regard to the cervical specimens, 69 were positive and 132 were negative by both test methods. For the 2 cervicals which were positive by the method of this invention but culture negative, the following information is available: one cervical came from a patient who was located, retested and found to be culture positive and again positive by the method of this invention. This patient was infected with N. gonorrhoeae which is apparently vancomycin sensitive because upon retesting that patient, N. gonorrhoeae was isolated from chocolate agar and Thayer-Martin medium which contains vancomycin. Because the test in accordance with the process of this invention is independent of any characteristic of N. gonorrhoeae infecting humans, other than ability to grow at about 36°C on chocolate agar in a CO_2 atmosphere, the method of this invention detected this positive the first time as well as the second time. In this regard, the method of this invention could be a very valuable test for the diagnosis of gonorrhea in areas where vancomycin sensitive strains of N. gonorrhoeae are common (Windall et al., J. Inf. Dis. 142:775, 1980). The second cervical came from a patient who was a contact to gonorrhea and had a positive rectal culture. This means that the patient had gonorrhea. In this case, the rectal as well as the cervical were positive by the method of this invention.

Based on this information, a reasonable conclusion is that there are no false positive tests in accordance with the process of this invention. The test is as specific for the diagnosis of gonorrhea as is the

culture method. The sensitivity of the invention test for cervicals, i.e., the number of positives detected is the same as for the culture method sensitivity of 97.3% (71/73).

With regard to the rectal specimens, 26 were positive and 128 negative by both test methods. Of the 4 rectals which were positive by the method of this invention but culture negative, all of the corresponding cervicals were culture positive as well as positive by the test method of this invention. Therefore these specimens came from patients who had gonorrhea. The four rectals in question were most likely false-negative cultures. Based on this information, the sensitivity of the method of this invention for rectals is 96.8% (30/31) compared to the culture method's sensitivity of 87.1% (27/31).

With regard to the urethral specimens, 74 were positive and 66 were negative by both test methods. Concerning the two urethrals which were positive by the test method of this invention but culture negative, both specimens were strongly positive for gonococci by the gram strain method. Therefore, the specimens came from patients who had gonorrhea. The two urethrals in question were most likely false-negative cultures. Based on this information, the sensitivity of the method of this invention for urethrals is the same as the culture method sensitivity of 97.4% (76/78).

In addition to the above data, 68 patients were tested for cures within 14 days after gonorrhea therapy. The results of both test methods were the same, i.e. the specimens from the patients were negative.

The method of this invention may be suitably performed by the use of a test kit. The kit consists essentially of an aliquot of a test strain of N. gonorrhoeae which grows poorly at about 36°C on or in a biological medium. The kit may also iclude (1) a solid

or liquid medium capable of supporting growth of the test strain, (2) a supply of a base, with or without ethanol, (3) a supply of an acid, (4) a pH indicator and (5) laboratory equipment or supplies. A test strain may be provided as a fresh culture or suspension, as a frozen suspension, as a lyophilized or dry preparation. The preferred biological medium is a chocolate agar or any other nutritional agar in any shape or form on which a test strain barely grows at a temperature around 36°C in a CO_2 atmosphere suitable for N. gonorrhoeae.

What is claimed is:

1. A preparation from a culture of the microorganism Neisseria gonorrhoeae ATCC 31953.

2. A preparation from a culture of the microorganism Neisseria gonorrhoeae ATCC 31953, said microorganism having characteristically poor growth on chocolate agar at a temperature within the range of from about 30°C to about 37°C in a CO_2 atmosphere suitable for growth of Neisseria gonorrhoeae, and said culture having specific resistance to nalidixic acid at the 5-10 mcg/ml level and streptomycin at the 1000 mcg/ml level or greater.

3. A preparation in accordance with claim 2, wherein said microorganism is competent to receive gonococcal DNA which imparts normal growth ability to the microorganism on or in a medium suitable for growth of N. gonorrhoeae at optimum N. gonorrhoeae growth conditions.

4. The method of detecting the presence of Neisseria gonorrhoeae DNA in a patients' specimen material or colonies suspected as being N. gonorrhoeae, whether in pure or mixed cultures, comprising the steps of (1) extracting DNA from the specimen or colonies by treatment with a base which lyses N. gonorrhoeae, (2) adjusting the pH of the extract of step (1) to a pH that is not toxic to a test strain of the microorganism Neisseria gonorrhoeae which has characteristically poor growth under conditions considered to be optimum for N. gonorrhoeae and is competent for transformation, (3) applying the pH adjusted extract to the test strain before or after putting it on or into a biological medium suitable for growth of N. gonorrhoeae, (4) maintaining the treated test strain on or in a medium suitable for growth of N. gonorrhoeae at optimum N. gonorrhoeae growth conditions which are inhibitory for the test strain, and (5) observing for detectable growth of the treated test strain.

5. The method of claim 4 wherein the pH adjusted extract of step (2) is heated at a temperature of from about 60°C to about 75°C for a time period from about ten minutes to about thirty minutes to inactivate remaining viable organisms prior to step (3).

6. The method of claim 4 wherein the pH adjusted extract is treated with ethanol to precipitate the DNA, which precipitate is then harvested and reconstituted in a small volume of a peptone containing buffer to effect a concentrated solution of DNA prior to step (3).

7. The method of claim 4 wherein the base comprises an ethanol-base mixture which simultaneously extracts and precipitates the DNA, which precipitate is then harvested and reconstituted in a small volume of a peptone containing buffer to effect a pH adjusted and concentrated solution of DNA prior to step (3).

8. The method of claim 4 wherein the base is an inorganic or organic base and the pH of the extract containing DNA is adjusted by an inorganic or organic acid.

9. The method of claim 8 wherein the pH adjustment is done in the presence of a pH indicator.

10. The method of claim 8 wherein the base is selected from the group consisting of NaOH, KOH or NH_4OH and the acid is HCl.

11. The method of claim 8 wherein the base and acid are prepared in a peptone containing buffer.

12. The method of claim 4 wherein the additional step of preparing a test strain of the microorganism N. gonorrhoeae which has characteristically poor growth under conditions considered to be optimum for N. gonorrhoeae and is competent for transformation is done prior to step (3).

13. The method of claim 4 wherein the test strain is maintained as a lawn on an agar containing medium suitable for the growth of N. gonorrhoeae at optimum growth conditions for N. gonorrhoeae but inhibitory to the test strain.

14. The method of claim 4 wherein the optimum N. gonorrhoeae growth conditions are a temperature of about 36°C, a suitable CO_2 atmosphere and a time period of from about 27 to about 40 hours.

15. The method of claim 13 wherein the medium is common chocolate agar.

16. The method of claim 15 wherein the chocolate agar contains an antibiotic at a concentration tolerated by the test strain.

17. The method of claim 16 wherein the chocolate agar contains an antibiotic selected from the group consisting of nalidixic acid and streptomycin.

18. The method of claim 4 wherein the test strain is in a form selected from the group consisting of a culture growth, a fresh suspension from a solid medium, a lyophilized or dry preparation or a thawed suspension of a frozen stock.

19. The method of claim 4 wherein the test strain is Neisseria gonorrhoeae ATCC 31953.

20. The method of claim 18 wherein the form of the test strain contains antibiotics tolerated by the test strain.

21. The method of claim 4 wherein the base and acid contain antibiotics at concentrations tolerated by the test strain.

22. A test kit for detecting Neisseria gonorrhoeae DNA consisting essentially of an aliquot of a test strain of Neisseria gonorrhoeae which grows poorly at about 36°C on or in a biological medium.

23. A kit in accordance with claim 22, wherein the test strain is N. gonorrhoeae ATCC 31953.

24. A kit in accordance with claim 22, characterized by the inclusion of one or more parts selected from the group consisting of (1) a solid or liquid medium capable of supporting growth of the test strain, (2) a supply of a base, with or without added ethanol, (3) a supply of an acid, (4) a pH indicator and (5) laboratory equipment or supplies.

25. Neisseria gonorrhoeae ATCC No. 31953.

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EUROPEAN SEARCH REPORT

0081078
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EP 82 10 9984

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
X	--- SEXUALLY TRANSMITTED DISEASES, vol.7, October/December 1980, American Veneral Disease Association, (US) L. ZUBRZYCKI et al.: "Laboratory diagnosis of Gonorrhea by a simple transformation test with a temperature-sensitive mutant of Neisseria gonorrhoeae", pages 183 to 187 * the whole article *	1-25	C 12 Q 1/04 C 12 Q 1/12 C 12 N 1/20 // C 12 R 1/36
D,Y	--- JOURNAL OF BACTERIOLOGY, vol.127, no.3, September 1976, American Society for Microbiology, (US) R.D. WHARTON et al.: "Temperature-sensitive mutants of Neisseria gonnorrhoeae", pages 1579 to 1581 * the whole article *	1-25	
Y	--- ABSTRACTS OF THE ANNUAL MEETING, 1975, American Society for Microbiology P. WARNER et al.: "Linking antibiotic resistance genes of Neissearia gonorrhoeae", page 97, column 1, abstract no. H6 * * the whole abstract *	1-25	
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TECHNICAL FIELDS SEARCHED (Int. Cl. ³)

C 12 N
C 12 Q
C 12 R
A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	07-02-1983	ENGELBRECHT E

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
A	CHEMICAL ABSTRACTS, vol.65, 1966, column 20544b, COLUMBUS, Ohio (US) P.F. SPARLING: "Genetic transformation of Neisseria gonorrhoeae to streptomycin resistance" & J. Bacteriol. 92(5), 1364-71 (1966) ---	2	
A	DIFCO MANUAL OF DEHYDRATED CULTURE MEDIA AND REAGENTS FOR MICROBIOLOGICAL AND CLINICAL LABORATORY PROCEDURES, ninth edition, Difco Laboratories, DETROIT, Michigan (US) pages 116 to 121 ---	13,14, 15	
A	DIFCO TECHNICAL INFORMATION, December 1976, Difco Laboratories, Technical Information no. 0580, DETROIT, Michigan (US) "Ready to use media for the isolation of N. gonorrhoeae or N. meningitides" -----	13-16	TECHNICAL FIELDS SEARCHED (Int. Cl. ³)

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